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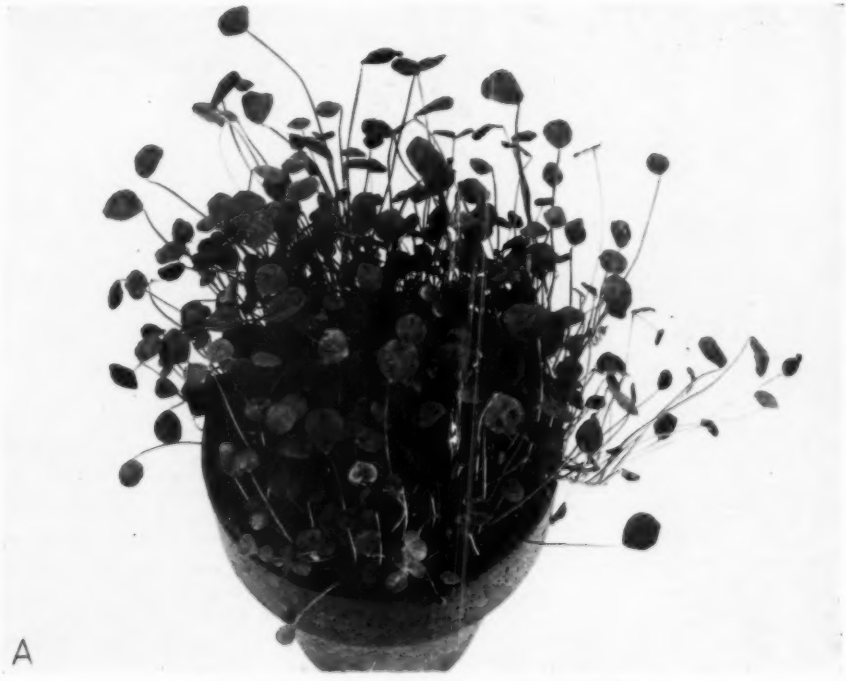
The Journal of HEREDITY



A

TRAITOR TO A TRIFOLIATE GENUS

(SEE PAGE 239)

Methods of Inducing Polyploidy

Thirty Million Dependents
vs
Thirty Million Solvent Families

The Ivory-Mutated Deer Mouse

The Hos Meet the Industrial Revolution

VOLUME 29 JUNE, 1938 NUMBER 6

The American Genetic Association

An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks

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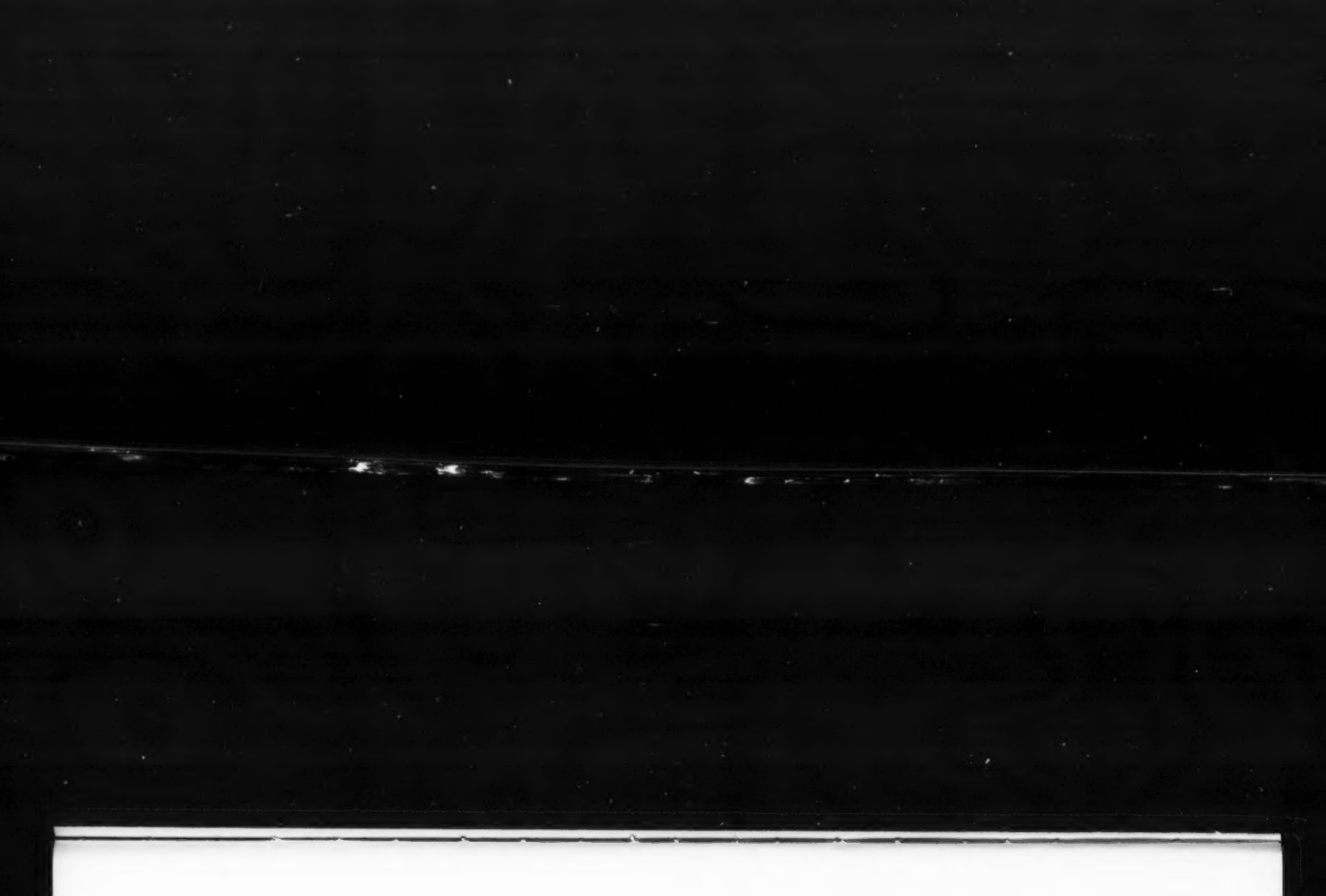

The

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EIGHT CELLS IN ONE

Frontispiece

The effect of colchicine on cell division and the multiplication of chromosomes within the cell is excellently shown by treating the stamen hairs of Rhoeo (Figure *2*) with dilute solutions of colchicine. This photograph shows two unaffected stamen hairs, made up of a single row of cells, like filamentous algae, and two greatly swollen hairs which have resulted from colchicine treatment. The effect of the colchicine was to stop the division of cells at the growing point of the hairs without stopping the division of chromosomes. Consequently all of the chromosomes which would have been distributed along the length of the hair in separate cells are congregated in a single giant cell. This process is somewhat modified in the upper affected hair, where the terminal cell has apparently four enlarged nuclei instead of one. In the lower cell there is only one gigantic nucleus, estimated to contain 96 chromosomes. This is eight times the normal diploid number in this species (*12*). Thus three cycles of chromosome splitting have occurred after treatment, each time with a doubling of the chromosome number, but without cell division. 750×.

A CYTOLOGICAL ANALYSIS OF POLYPLOIDY

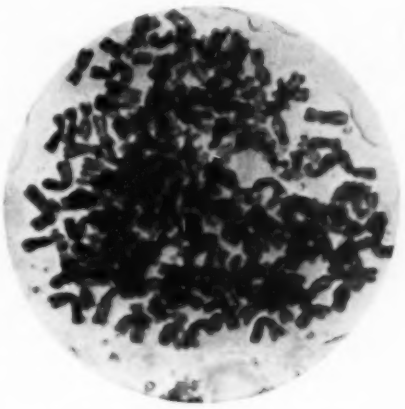
Induced by Colchicine and by Extremes of Temperature*

HAIG DERMEN

Bureau of Plant Industry, U. S. Department of Agriculture

THE subject of polyploidy has become of keen interest recently because of the development of methods by which it can be artificially induced. The work of Randolph[7] in inducing tetraploidy in maize by means of *heat treatment*, and more recently the reports of Blakeslee and Avery,[2] and of Nebel and Ruttle[6] on the use of the drug *colchicine* in inducing polyploidy, indicate methods by which increase in the number of chromosome sets may be induced in certain plants. For a discussion of the subject of polyploidy in plants, as to its value in agriculture and significance in evolution, the reader is referred to the excellent discussions by Anderson[1] and Muntzing.[5]

The interest in the artificial production of polyploidy is justified, since Munzing concludes that more than half the species of flowering plants in which the chromosome number is known are polyploids. These comprise a large portion of our economic plants. If so many of the natural polyploid plants are recognized as being especially valuable, it may be assumed that important results might be obtained by artificial induction of polyploidy in plants already recognized for their value. Though these new methods are promising, plant breeders should be cautioned not to expect success in all attempts. Genetic constitution and physiological balance are factors that will determine the size and the function of an organism, the success of experiments, and the value derived from them.



FROM FOUR CHROMOSOME DOUBLINGS

Figure 1

A somatic chromosome metaphase plate from material treated with 0.5 per cent colchicine. The chromosome number is estimated to be 192, which represents four doublings of the diploid chromosome complement of 12. It appears that colchicine does not affect chromosome growth or the ability of the chromosomes to divide. Rather it inhibits the mechanism which normally pulls apart the split halves of the dividing chromosome, separating them into two equivalent nuceli. Magnified 750 times.

The observations reported here on artificially induced polyploidy were made on *Rhoeo discolor*, a species of the spiderwort family (Commelinaceae),

*I wish to thank Mr. E. L. Green, associate biochemist at this station, for helpful discussion of some of the physico-chemical problems involved in these experiments. For careful reading and criticism of the manuscript, I wish to express my appreciation to Dr. J. R. Magness, Dr. L. F. Randolph, Dr. M. M. Rhoades, and many of my colleagues at this station.

A PLANT "GUINEA PIG" FOR COLCHICINE EXPERIMENTS

Figure 2

Rhoeo discolor has many advantages for cytological studies on methods of inducing polyploidy. Under greenhouse conditions within a considerable range of temperature (16°-30°C.) Rhoeo remains in bloom all the year around. It has only six pairs of chromosomes which are about as large as those of Tradescantia. It breeds true from seeds and it may also be easily propagated from small shoots which grow on the soil level from the stem of the plant. Vegetative effects of the polyploidy treatments can be studied from the hairs growing around the basal portion of the anther stalks, while the effects on the sexual cells can be studied from anthers which are easily handled in smear preparations.

which has 12 ($2n$) chromosomes. Since Rhoeo had been used (Sax[8,9]) in studies on the mechanism of polyploidy as affected by temperature changes, it was selected for similar work with colchicine and was found to be ideally adapted to this type of experiment. Presumably the principles involved in induction of polyploidy in Rhoeo may readily be applied in experiments with economic plants. Some additional data concerning the effect of temperature changes on Rhoeo which were obtained by the author will also be presented here.

In this report an attempt is made to interpret and evaluate the cytological phenomena involved in artificially induced polyploidy brought about by these two methods in mitotic as well as in meiotic phases. Pertinent cytological phases are illustrated in 36 photomicrographic reproductions. Figures 4 to 5 are from colchicine-treated material, and Figure 6 illustrates material subjected to temperature changes. Differences, as well as similarities occurring in the effects of these two methods, are indicated.

At the end of the text a few suggestions are presented that may be helpful to plant breeders who are interested in performing such experiments.

The cytological data on polyploidy induced by temperature changes were obtained at the Arnold Arboretum of Harvard University between the years 1935 and 1937, while the experiments with colchicine were started at the U. S. Horticultural Station at Beltsville, Maryland, in January, 1938.

DETAILS OF FLOWERING HABIT
Figure 3

The flower buds of Rhoeo grow in very compact clusters in which all phases of development may be found. As the older buds bloom and mature, younger buds are continually developing in the same cluster. Ordinarily two flowers mature in such a cluster each day, so that a few plants will give an abundance of cytological material.

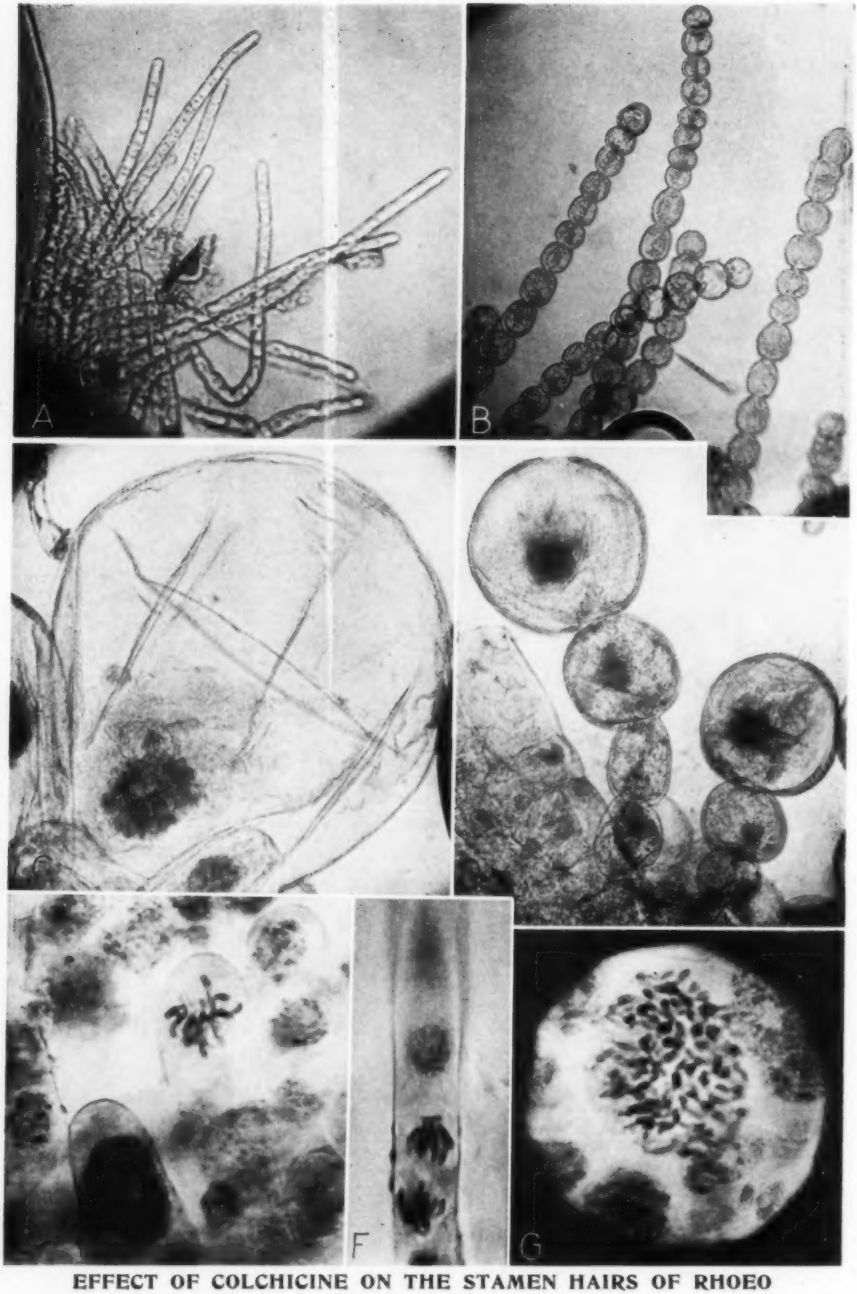
Material and Methods

Observations were made on both somatic and meiotic phases of the flower of *Rhoeo discolor*. Special attention was given to the colchicine effect on stamen hairs and on meiotic cells in all stages of their development. The hairs are filamentous, made up of cells ranged end to end, like filamentous algae when young (Fig. 4*A*); and are like a string of beads in mature hairs (Fig. 4*B*).

Rhoeo flowers grow in two opposite rows in clusters, partly enclosed by two or three short bladelike bracts. In addition to these bracts there are a number of papery thin scales that cover up the young buds. For colchicine treatment the bracts as well as scales were carefully removed to expose the young buds as much as possible without injury to them. Then aqueous solutions ranging from 0.1 per cent to 1 per cent were applied to different flower clusters by wetting the buds thoroughly with the aid of a camel's-hair brush. The buds treated without first removing bracts and scales showed no irregularities comparable to those treated with bracts and scales removed. Control buds that were similarly exposed developed normally. In some cases, there was added to the colchicine solution an equal amount of 0.2 per cent Aresket* to facilitate the spread of colchicine solution over and between flower buds. The regular use of this method, however, was limited to other plants which were tomentose.

A number of tests involving temperature changes were made in order to determine what specific treatments were most effective. These tests were made only to study changes in meiotic phases. Five separate treatments were given: (a) cold, (b) heat, (c) cold followed by heat, (d) heat followed by cold, and (e) cold followed by heat treatment and a return to greenhouse temperature for two days, and then a second cold treatment. The greenhouse temperatures ranged from 18° to 28° C. For cold and heat treatment, two glass boxes with temperature controls were used. The cold chamber was kept between 5° and 10° C., and the heat chamber between 32° and 35° C. A greenhouse cold pit was also used, where the temperature ranged between 5° and 10° C. The length of time for these treatments varied from 12 to 24 hours. At the above temperatures, an exposure of six hours was tried, but the effect was lim-

*A wetting agent used in spraying by orchardists. This method was suggested by Mr. E. L. Green, associate biochemist at this station. The chemical make-up of Aresket may be found on page 959, second paragraph under *Material and Methods*, in the article by Green and Goldsworthy[3].



EFFECT OF COLCHICINE ON THE STAMEN HAIRS OF RHOEO

Figure 4

Normal young stamen hairs (*A*) and normal mature stamen hairs (*B*) of *Rhoeo discolor*. These are from untreated material, and show the development of the stamen hairs as single

ited, and only a few of the pollen mother cells were affected in a given anther.

A Zeiss microscope with a 20× apochromat objective and a 30× compensating ocular were mainly used and a Bausch & Lomb "Type K" camera was used in taking the photomicrographs.

Results

Somatic changes from colchicine treatment.—The effect of colchicine was apparent in all parts of the flowers. The effect on stamen hairs, however, was the most striking.

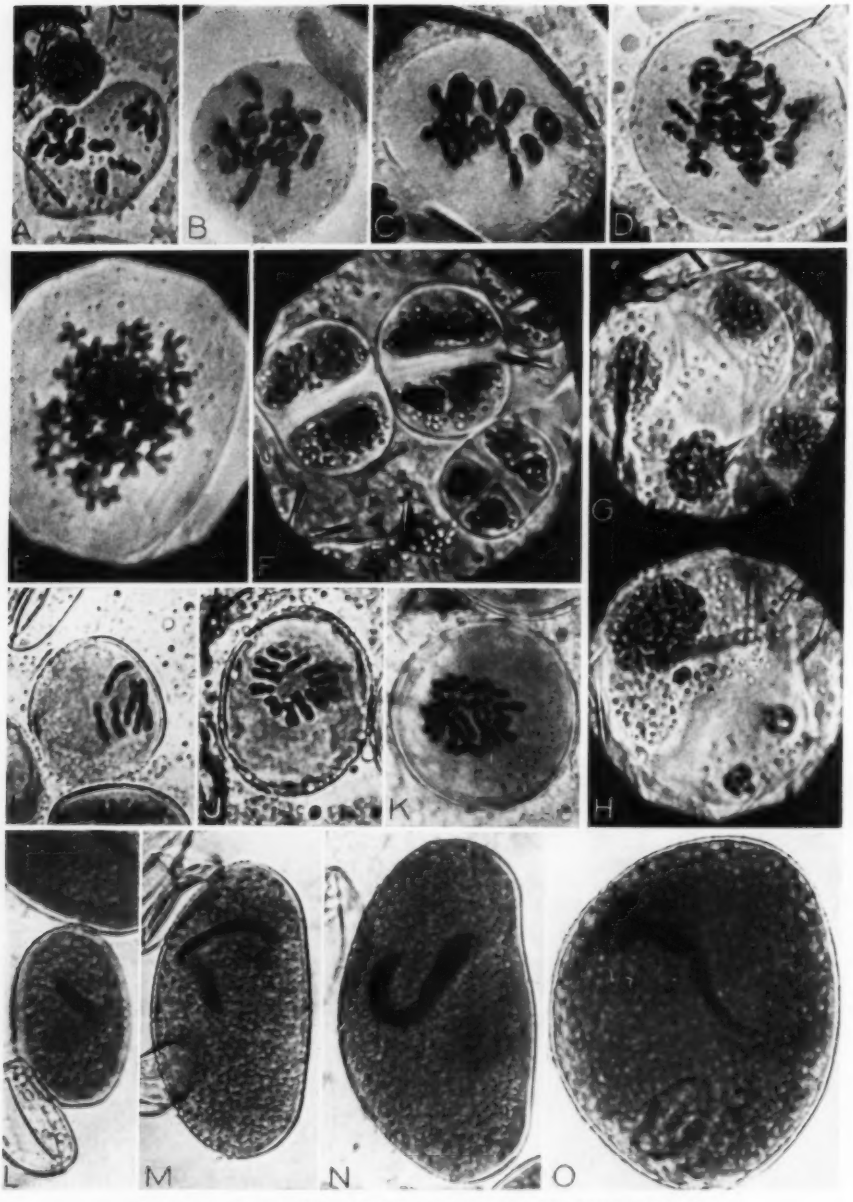
1. Gross observations on the stamen hairs.—Normal hairs are shown in Figures 4*A* and 4*B*, the first being a young stage and the second a mature stage. In the mature hairs the cells are rounded out and appear like a string of beads, numbering between 30 and 50 to a hair. In the young stage shown in 4*A*, the hairs contain between 15 and 20 cells. In the still younger stage, division may go on in all cells of the hair, but the rate of division is higher in those toward the end and in the end cell. The buds from which Figures 4*C* and 4*D* were taken were treated with a 1 per cent colchicine solution and collected six days after the treatment. The bud from which Figure 4*C* was made was comparable to that of Figure 4*A* and the bud of Figure 4*D* to that of Figure 4*B*. The hairs in the material of Figure 4*C* were almost all only one cell long, but they were extremely large. The nucleus, as well as the cell as a whole, had increased in volume. In all cases there was an obvious relationship between nuclear and cell volume, although in some cells there was apparently a greater increase in cell volume than in nuclear volume. In the material illustrated by Figure 4*D*, the number of cells to a hair ranged from two to eight. In all cases, the end cell was larger than those below it. The gradation in size held true from tip to basal cells.

2. Cytological observations on the stamen hairs.—The material illustrated in Figures 4*E* and 4*F* was treated with a 0.2 per cent colchicine solution and collected eight days after the treatment. Some of the hairs were apparently affected by the drug, while others had escaped the effect. Figure 4*E* shows an unaffected hair, as yet but a single cell long, containing a normal number of chromosomes as seen from side view in metaphase stage. An affected hair, also a single cell long, is shown just below the normally developing hair, and contains a nucleus about four times the normal volume. Figure 4*F* illustrates the normal division of somatic chromosomes. The upper cell has just completed the division of a nucleus into separate daughter nuclei, which are rounded up and which will later be separated by a wall, while in the cell below the chromosomes have just divided and moved apart. Normally they would soon go into the resting stage and complete the cycle of nuclear as well as of cell division.

The scattered effect of colchicine is strikingly illustrated in the Frontispiece, also from material collected eight days after treating with 0.2 per cent solution.

Legend to Figure 4—Continued

alga-like strands made up of a bead-like string of cells. The number of cells in the mature stamen hairs varies between 30 and 50. The five other photographs here shown are of material treated with colchicine. The effect of the treatment on the stamen hair depends on the point in the growth history of the hair at which the colchicine came into contact with the hair. *C* shows a single hair cell from material treated with colchicine (1%). Cell division has been entirely inhibited and an enormously ballooned single cell has resulted. *D* shows hairs 6-8 cells long in which the colchicine (1%) acted after some of the basal cells had developed. *E* shows how colchicine affects adjoining hairs differently. Three hair-primordia from treated material (0.2%) are shown. The one at the center is at mitotic metaphase, and apparently normal, as is that at the upper right. The one below has been affected by the drug, having a nucleus abnormally large. *F* shows an unaffected hair from treated material (0.2%), with normal mitotic chromosomes distributed toward opposite poles. *G* shows a late prophase of an ovular cell with chromosome number estimated at 48 ($4 \times 2n$) from material treated with 0.2% colchicine. *A-B* 125×, *C-G* 750×.



EFFECT OF COLCHICINE ON POLLEN FORMATION

Figure 5

A—diploid (2*n*) pollen mother cell with 12 univalent (unpaired) chromosomes from material treated with colchicine (0.5%)-aresket (0.2%) solution. Ordinarily the diploid pollen mother cell contains only 12 chromosomes in six pairs. *B-C*—4*n* pollen mother cells from material treated with 0.2% colchicine. The former (*B*) with the chromosomes partly univalent but mostly in the form of a chain. The latter with the chromosomes in 12 separate

There are shown two unaffected hairs about like the one in Figure 4*F*, and two others showing a striking increase in size of cells. The upper hair appears to be multinucleate with larger nuclei than normal, while in the one below there have been three doublings of the chromosome number, which is estimated to be 96, eight times the diploid number, 12. The chromosomes are at the metaphase stage and morphologically all appear to be at the same stage of development. The chromosome plate happened to be on a slant, and it was not possible to show all the chromosomes present clearly. The lower cells of this particular hair show size increase, where perhaps the nuclei have doubled only once or twice.

3. Stamen tissue.—Cells of both the filaments and anther walls showed the effect of colchicine. Figure 1 is from anther tissue from buds treated with a 0.5 per cent solution and collected eight days after treatment. The anthers were fixed in Carnoy's solution, smeared for a study of meiotic stages, and stained with aceto-carmine. In the preparation, a few chromosome plates were found similar to the one in Figure 1. The number of chromosomes in this figure is estimated to be 192, 16 times the $2n$ number or 32 times the n number. There were some other flattened nuclear masses, in which it seemed that the number of chromosomes reached twice the above number, or a total of 384 chromosomes, through a doubling five times of the 12 original chromosomes. This number could be derived from the somatic number 12 by repeated division without the separation of the sister chromosomes.

4. Ovular tissue.—A 0.2 per cent solution of colchicine not only affected stamens and stamen hairs, but also ovary tissue, penetrating it and reaching the ovular tissue, where there was a decided increase in nuclear and cell volume. Such an effect is shown in Figure 4*G*. Here the chromosomes are still in prophase stage, but the number was estimated to be 48.

5. Cuticular inflation. — In all colchicine treatments, what appears to be cuticle showed in part, or wholly, an inflated appearance around the stamen filaments. It appeared as if the cuticle had been separated from the epidermal layer and had expanded in a continuous sheet into a cylinder around the filament. Some of the stamen hair cells were affected similarly. In the hair cells where such inflation was observed, the cell wall seemed to have separated into two sheets—an outer and an inner. The inner part alone kept the cell volume, as well as the shape, unchanged.

6. Observations on the treated flower buds as a whole.—In most of the treatments the effect of colchicine on young flower buds was obvious. The flower stalks grew stockier and shorter than normal, and some buds were almost

---

pairs. *D* an $8n$ pollen mother cell with part of the chromosomes unpaired and part of them in chains (0.2% Col.). *E* a $16n$ pollen mother cell, where the chromosomes may be dividing homotypically, but in which the dividing chromosomes may fail to separate into two groups (0.5% Col). *F-H*—Tetrads of pollen mother cells (P.M.C.) (0.2%). *F* shows one small cell derived from a $2n$ P.M.C. The two large ones are derived from $4n$ P.M.C.'s. *G* and *H* are from $8n$ P.M.C.'s. The four nuclei shown in *G* are of about the same size but those in *H* are unequal in size, with one very large nucleus and one very small just below it,—the other two nuclei being equal size. Such unequal nuclei are the result of unequal reductional and homotypic divisions. *I*—a normal haploid microspore with six chromosomes, notwithstanding that the material was treated with one per cent colchicine. *J*—a diploid microspore with 12 chromosomes from the same material as *I* but affected by the colchicine treatment. *K* is a tetraploid microspore with 24 chromosomes from the same material as *I* and *J*. The lower row (*L-O*) shows pollen grains having various chromosome complements. *L* is a normal haploid pollen grain derived from material treated with aresket-colchicine solution. *M* is a diploid pollen grain; *N* is a tetraploid; and *O* is a giant pollen grain either octoploid or with a few chromosomes less than the full $8n$ complement. All of these pollen grains are from material treated with a solution made up of one-half aresket (0.2%) and one-half colchicine (0.2%). All figures are magnified 750×.

stalkless. A similar type of effect was noticed on petals, which were stunted and coarsened in texture. The filaments were short and the hairs were completely eliminated or merely grew into unicellular structures with large dimensions, as is shown in Figure 4*C*. When the bud primordial cells were affected, flower buds ceased to be developed. When very young buds were affected, the anthers were apparently filled with large cells and contained no meiotic cells. If such anthers were smeared, only thin liquid was exuded. With higher concentrations of colchicine, the whole flower cluster eventually died.

Meiotic changes from colchicine treatment. — Practically all the important phases of microsporogenesis from colchicine-treated material that were observed are illustrated in Figure 5. Meiotic chromosomes in Rhoeo normally join end-to-end in pollen mother cells and form either a complete ring of 12 chromosomes (Figure 6*A*) or a continuous chain (Figure 6*B*) or broken chains, and never associate in six pairs.

1. Changes in pollen mother cells.— In a preliminary test using 1 per cent solution, it was observed that the normal development of tetrads in pollen mother cells had been upset, and diads, monads, and many irregularly divided "tetrads" were found. Buds from the same cluster in an earlier stage of development at the time of treatment failed to show typical reduction and homotypic division in the pollen mother cells. It was apparent that meiotic chromosomes had gone into a resting stage, without undergoing the usual anaphasic separation into two groups. The ring or chain of chromosomes had broken apart during what corresponds to the interphase between divisions I and II and chromosomes had reappeared for the homotypic division. By this time each chromosome had split, the whole chromsome group again had gone directly into resting stage, and then began the development of the microspore stage. The end result was a single cell with the tetraploid number of chromosomes rather than a tetrad of microspores, each with a haploid set of chromosomes. Beginning about the fourth day after the treatment, meiotic chromosomes appear in shorter broken chains or in univalent form. Five or six days following the application of 1 per cent solution, there were among $2n$ pollen mother cells a number of $4n$ pollen mother cells containing univalent chromosomes. Figure 5*A* illustrates a pollen mother cell that was fixed four days after the application of equal parts of Aresket, 0.2 per cent, and of colchicine, 1 per cent solution. Here the meiotic chromosomes are in univalent form. To one of the chromosomes at the lower right a piece of chromosome is attached which is about the same size as what corresponds to the short arm. Whenever meiotic chromosomes were univalent, the reduction division was never observed.

The material illustrated in Figures 5*B* to 5*D* was treated with 0.2 per cent solution, and the bud from which 5*E* was taken was treated with 0.5 per cent solution. They were collected and fixed eight days after treatment. In this preparation the anthers at meiotic stage contained only $4n$ and $8n$ pollen mother cells; the $8n$ pollen mother cells made up the large bulk of the cells. Some of these probably contained some or all univalent chromosomes, as may be the case in the pollen mother cell shown in Figure 5*B*. Rarely there were $4n$ cells where the chromosomes had paired in twos, as indicated in Figure 5*C*. Such a pairing of chromosomes is contrary to normal behavior in $2n$ pollen mother cells where the chromosomes join end-to-end and usually form a closed ring, although it was expected that in $4n$ pollen mother cells, pairing in twos should have been most prevalent since due to a somatic doubling of chromosomes each chromosome had now an entirely homologous mate to pair with, while in $2n$ they are supposed to be only in part homologous, hence the end-to-end pairing in the form of a ring or a chain. Figure 5*D* represents an $8n$ pollen mother cell where apparently most of the chromosomes are univalent and a few may have paired. Figure 5*E* rep-

resents a 16*n* pollen mother cell in which the chromosomes have divided, but it seems likely that the process of chromosome duplication would have been repeated once more.

2. colchicine effect on microspores derived from affected pollen mother cells.—Depending upon the stage of development of the pollen mother cells at the time colchicine was applied and entered these cells, we find either irregularly shaped young microspores containing more than one nucleus, or uninucleate microspores with the nucleus double the normal volume. Some of the microspores seemed to have retained the diploid number of chromosomes but showed partial division of the cell by the furrowing method, the part on one side of the furrow being devoid of nuclear matter. Some anthers contained almost entirely diploid size microspores, while others were completely filled with tetraploid microspores. The actual haploidy or polyploidy of affected microspores may be confirmed at the first nuclear division which follows about 11 days after meiosis.

The metaphase stage of the first nuclear division of three types of microspores is shown in Figures 5*I* to 5*K*. These were from material treated with 1 per cent solution and collected 12 days after treatment. The first one with six chromosomes is a haploid microspore, the second with 12 chromosomes a diploid, and the third with 24 chromosomes a tetraploid. Both the haploid and the diploid microspores are somewhat oblong, while the tetraploid microspores are entirely round, indicative perhaps of their source of origin. These colchicine induced tetraploids originate from the complete failure of both reductional and homotypic divisions, the diploids from the failure of homotypic division, while haploids originate from normal tetrads. From this material no mature polyploid pollen grains were obtained, perhaps because the first nuclear divisions in these microspores failed to occur due to the continued presence of colchicine in these cells or due to the toxic effect of colchicine on the microspores at higher concentrations.

3. Microspores derived from microsporogenous tissue affected before meiotic differentiation.—As a result of somatic chromosome doubling and redoubling in the microsporogenous tissue of very young anthers, as was indicated above, polyploid pollen mother cells were obtained. These are shown in Figures 5*B* to 5*E*. In some material treated with 0.2 per cent colchicine concentration, such pollen mother cells may behave quite normally and meiotic chromosomes may divide reductionally as well as homotypically. In Figure 5*F* are shown three tetrad pollen mother cells; one is small and the other two are large. The small one represents haploid microspores derived from a normal diploid pollen mother cell, while the other two represent diploid microspores derived from tetraploid pollen mother cells. Figure 5*G* is a tetrad from an octoploid pollen mother cell (this cell was pressed down in the preparation). Figure 5*H* (also from pressed material) represents a tetrad stage also derived from an octoploid pollen mother cell, but due to an uneven reductional as well as homotypic division there is one large nucleus and three much smaller nuclei. The one nucleus just below the large one may contain only one chromosome and the other two small nuclei two or possibly three chromosomes. The large nucleus may contain about 45 chromosomes, three less than the octoploid number.

4. Pollen grains resulting from colchicine treatment.—Pollen grains resulting from treatment may be divided into three classes: (1) pollen grains from mother cells that were affected either at or just before the reduction division or at homotypic division; (2) pollen grains resulting from mother cells that were affected before meiotic chromosomes had paired (leptotene stage); and (3) pollen grains from polyploid pollen mother cells derived from premeiotic microsporogenous tissue affected by colchicine.

Four different sizes of mature pollen grains are shown in Figures 5*L* to 5*O*. Figure 5*L* represents the normal haploid

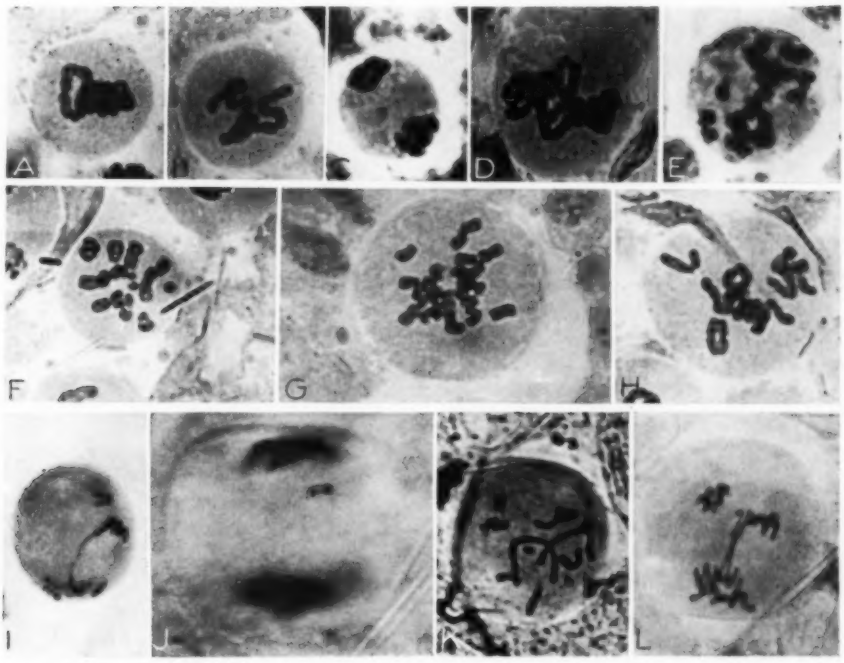
type, Figure 5*M* the diploid type, Figure 5*N* the tetraploid type, and Figure 5*O* possibly a truly octoploid pollen grain or one lacking perhaps a small number of chromosomes. The material from which these pollen grains were obtained was treated with a solution consisting of equal parts of 0.1 per cent Aresket and 0.1 per cent colchicine, and the pollen grains were collected 22 days after treatment. The long, darkly stained part in these grains is the generative nucleus, while the tube nucleus hardly shows in the photomicrograph, although it was present in the pollen grains. No mature pollen grains were obtained from buds treated with either 1 per cent or 0.5 per cent colchicine.

Polyploidy in the meiotic phase of Rhoco discolor induced by temperature changes. — In the experiment with changes of temperature from optimum to low or high, observations were made only on meiotic phases in Rhoeo. The observations here reported will be primarily concerned with doubling of chromosomes in premeiotic tissue of the anthers and the phenomena of chromosome fragmentation and fusion in the treated material.

With sudden changes from greenhouse temperature to either cold or warm, diploid pollen grains were obtained. In order to find out how sudden these temperature changes had to be to induce polyploidy, two preliminary experiments were conducted. In one, some plants were put in a cold-treatment box which had started with the same temperature as that of the greenhouse (15° C.). Then cold air was turned on. Within five hours the temperature had dropped to 4° C. This temperature was maintained for 18 hours. The cold-air current then was turned off, but the door of the box was left shut and the plants were kept in it until the inside temperature had reached the outside temperature. This took about 12 hours longer, at which time both the greenhouse and the box temperatures were at 16° C. Similarly, some plants were treated with a gradual change to high temperature, and the result, so far as inducing polyploidy is concerned, was the same as when the temperature change was made abruptly.

All the illustrations represented in Figures 6*A* through 6*H* are from the experiment in which the plants were transferred from greenhouse temperature to cold air and from cold air to greenhouse temperature gradually. The material illustrated in Figures 6*A, B, D, F, G* and *H* was fixed ten days after treatment and that in Figures 6*C* and 6*E* 13 days after treatment. Figures 6*I* to 6*K* were from material transferred from greenhouse temperature at 26° C. to the cold chamber at 13° C. and kept there for 18 hours, then transferred to the heat chamber at 33° C. and kept there for 25 hours, and then transferred back to the greenhouse at 22° C. These illustrations were made from material fixed 12 days after treatment. Figure 6*L* was from a plant first put into the greenhouse pit at a temperature of 8°-10° C. for about 22 hours, then into the heat chamber at 33° C. for 24 hours, and back to the greenhouse at 21° C. Two days after the cold-heat treatment, these plants were again put into the cold pit at 12° C. for 18 hours and back to the greenhouse at 25° C. This last temperature change was to induce redoubling in meiotic cells dividing homotypically, which had been previously prevented from dividing reductionally, to produce 4*n* pollen grains. The material of Figure 6*L* was fixed eight days after the last treatment.

Figure 6*A* shows the mode of pairing of meiotic chromosomes in Rhoeo. The chromosomes either form a closed ring by end-to-end, zigzag attachment or a continuous chain (Figure 6*B*) or rarely broken chains. Figure 6*C* is the telophase stage after a reductional division. Figure 6*D* represents a tetraploid pollen mother cell derived from the doubling of somatic chromosomes in a cell previous to meiotic differentiation. The number of tetraploid pollen mother cells thus derived makes up 1 to 3 per cent of the meiotic cells; all the rest are diploids. The pairing of meiotic chromosomes in these 4*n* cells is usually in



POLLEN FORMATION AFFECTED BY HEAT AND COLD TREATMENTS

Figure 6

A and *B*—two normal 2*n* pollen mother cells (P.M.C.'s), showing the chromosomes in a closed ring (*A*), with the chromosomes in a chain form (*B*). At telophase (*C*) the two chromosome groups separate and go to the opposite poles. *D*—a 4*n* pollen mother cell derived from low-temperature treatment by gradually decreasing the temperature from optimum (15°C) to low (4°C). The chain formation is normal as can be seen by comparison with *A*. *E* shows a regular reduction division of the 4*n* P.M.C. *F*—a diploid pollen mother cell (from same material as *D*) showing fragmentation and possibly fusion of chromosomes. Fragmentation and fusion are found in temperature treated material but not in colchicine treated material. Additional fragmentation is shown in *G*, a tetraploid P.M.C. from the same material as above, showing some fragments among unaffected chromosomes, some univalents and some chromosomes paired end-to-end in a chain. *H* shows another tetraploid P.M.C. from this same material with chromosomes joined in broken chains. *I*—haploid microspore chromosomes at late anaphase of first nuclear division, showing a single-line chromatin bridge, indicating that fusion has occurred from material treated by cold followed by heat. *J* shows haploid microspore (1240×) chromosomes at telophase, showing a chromosome fragment split but not separated. From material treated the same as in *I*. *K*—diploid microspore chromosomes at metaphase of the first nuclear division, with chromosome fragments and fused chromosomes. From material treated as in *I*. *L*—a diploid microspore with chromosomes at late anaphase showing cross-line chromatin bridge. From material treated by cold followed by heat; this followed by transfer to room temperature; and again to cold, and then back to room temperature. This cross-bridge is evidence that through the treatment a translocation has occurred. All figures 750×, except *J*.

broken chains (Figure 6*H*) or some times in a single large, closed ring (Figure 6*D*), in which the exact alignment of chromosomes is difficult to follow. They may remain almost entirely univalent as shown in Figure 6*G*, an which there seems to be a limited amount of chromosome association. What few tetraploid pollen mother cells could be found in first metaphase

showed no case where chromosomes paired entirely in twos, as shown in Figure 5*C*, only once seen in colchicine-treated material. It was found that apparently, when chromosomes pair in a more or less continuous chain or a closed ring, the reduction division is quite normal as in the diploid (Figure 6*C*). When, however, chromosomes are univalent there is a complete failure of reduction division such as occurs in the diploid.

Besides doubling of chromosomes, there was present a good deal of fragmentation and fusion in the chromosomes both in cold- and heat-treated material. These may have occurred during either the premeiotic stage or the meiotic stage. Fragments were found in diploid (Figure 6*F*) as well as tetraploid (Figure 6*G*) pollen mother cells which were derived from material that was affected during the premeiotic stage. These same cells may also have had fused chromosomes, but that can not be detected in Rhoeo—where chromosomes join end-to-end—unless they divide reductionally and show chromosome bridges between the two poles of the divided chromosome groups. Such bridges were found in abundance in all temperature-treated material, and were especially apparent in cold-heat-treated material that was studied two days after it was treated. Fragments were usually observed at the metaphase of the first nuclear division in both haploid and diploid microspores. Similarly, fused chromosomes seemed to be always associated with such fragmentations. Such occurrences were few in haploids and were very frequent in the diploid microspores at this particular stage. Figure 6*I* shows a bridge in the haploid microspore resulting perhaps from a fusion between two daughter chromatids of a chromosome. In Figure 6*J* (highly magnified) a fragment is shown which is partly divided and, perhaps because of having no spindle point, has failed to pass to either pole. Figure 4*K* shows a diploid microspore with a number of fragments and two long, fused, compound chromosomes. Such diploid microspores, in which the length of fused chromosomes was variable, were quite abundant in this material. Similarly, the length and width of fragments were variable, some being perhaps chromosome fragments and some chromatid fragments. As a result of fusion, chromosomes will often possess more than one spindle region, usually two. When in the microspores such fused chromosomes divide, they often form two chromatin bridges (Figure 6*L*) which criss-cross. In Figures 6*I* and 6*L*, striking types are shown which demonstrate the presence and the type of fragments and bridging of chromosomes as a result of temperature changes from optimum to sufficiently low or sufficiently high.

Discussion

There are a number of factors that are known to affect the mechanism of cell division and induce chromosome doubling (See Sax[10]). Of these factors, until recently, temperature was the most promising, since it was successfully used in producing polyploid plants instead of merely polyploid cells or sectors in a plant. Randolph[7] was perhaps the first to succeed in developing polyploids by using heat treatment technique. He treated maize with high temperature and induced tetraploidy in the developing embryos. Kostoff[4] introduced another method, that of centrifuging germinated seeds, and produced a tetraploid Matthiola plant. Similarly, from a seedling of *Nicotiana rustica* × *N. tabacum* (a sterile hybrid), a fertile branch was developed that had the double number of chromosomes. Since Randolph's work on maize a number of other investigators have been able to produce polyploid plants by applying the temperature technique.

Just recently, by the discovery of the use of colchicine, a new method has been developed that promises to be of wide use in the production of polyploid plants in larger percentages than any other methods so far known. This was indicated by the work of Blakeslee and

Avery[2], and Nebel and Ruttle.[6*] Colchicine may also be used in the study of the mechanism of cell division and other problems associated with this mechanism.

Our immediate interest lies in a study of the most outstanding phenomenon relative to the application of colchicine in inducing polyploidy in plants. This is the high percentage of polyploids obtained from the use of this drug. Blakeslee and Avery[2] report that by treating seeds of Datura with colchicine they obtained as high as 85 per cent tetraploid plants, judging from the appearance of the plants, or 65 per cent tetraploids as determined by pollen-grain size. By heat treatment, on the other hand, no one as yet has been able to obtain more than five per cent polyploid plants (Randolph, from a seminar report).

Polyploidy by temperature change.—In *Rhoeo discolor*, temperature changes seem to affect both meiotic and somatic chromosome distribution. Both with low and high temperature treatments, meiotic chromosomes at the time of first metaphase seemed to be forced together; therefore, the immediate effect of this treatment seems to be to bring about clumping of chromosomes, and presumably this disrupts the mechanism controlling nuclear as well as cell division. A lowering of viscosity of the cytoplasm due to cold and heat treatments was observed in meiotic cells when the anthers were smeared and meiotic cells were flattened on the slide during the staining of the treated material. These cells were disrupted much more easily and were more fluid in appearance after treatment than before. However, after nuclear as well as cellular division was resumed, interphase and tetrad cells still showed the same fluidity and were also disrupted easily. This may perhaps indicate that if the lowering of viscosity in cytoplasm has a role in the disturbance described here, this effect occurs only when the viscosity change is at its maximum.

When the temperature is raised or lowered leptotene chromosomes may either precociously shorten or they may not lengthen sufficiently, thus preventing the joining of chromosomes by their homologous parts. When such chromosomes appear at later stages of the reductional division they appear as univalents. On account of univalency of the chromosomes in Rhoeo, the mechanism of both nuclear and cellular division either fails to appear or is rendered non-functional, and the chromosomes all together go into resting stage, and at their reappearance they divide only once homotypically. Whenever there is a partial pairing in these chromosomes, the paired ones become polarized and, eventually, joined chromosomes are set in motion toward opposite poles, while the univalents are rendered neutral and as a result the division becomes uneven and various-sized nuclei may form in the pollen mother cells.

Due to a high or low temperature, *first*, meiotic chromosomes fail to segregate and may only divide homotypically; *second*, if the effect of temperature is on chromosomes that have already been segregated and are dividing homotypically, this division may be prevented and the resulting cells may directly develop into microspores. In either case, we may obtain only diads. However, these diads genetically are dissimilar. In the first instance, because reduction has been prevented, we may obtain diads that are genetically homogeneous, unless crossing-over between homologous chromatids has taken place when chromosomes were paired; while in the second, because the first division was reductional, the diads are of two classes. The diads obtained on account of univalency will be of one class, since there had been no pairing of chromosomes and no crossing-over could have taken place. In

*Blakeslee and Avery credit Dr. O. J. Eigsti for suggesting to them the use of colchicine for this purpose, and Nebel and Ruttle state that Dr. D. F. Jones mentioned to them the possible value of the drug for this purpose. The experiments with colchicine by these authors were conducted almost simultaneously but independently.

cases where plants are re-treated or treated first with cold and then with heat, or vice versa, after reduction has been prevented, often the homotypic division may also be disturbed. In this way, tetraploid microspores may be produced that are also entirely homogeneous. In plants where abortion or sterility of pollen grains is controlled by genetic factors, such phenomena may be suppressed or the percentage of such pollen grains may be modified by polyploidy of pollen grains.

The disturbance effected by high or low temperatures on chromosomes and dividing cells may be only temporary and may be eventually overcome, but microspores which are young at the time of the treatment seem to be permanently affected and the great mass of them will be destroyed. Thus, until new microspores develop and reach maturity in the anthers of younger buds, anthers in the older buds will be found mostly devoid of pollen grains.

As a result of temperature treatment, at about the eleventh day after treatment and for four or five days longer, there will be an abundance of diploid microspores at metaphase of the first nuclear division. During this interval most of the anthers will contain only diploid microspores.

It was shown above that in the temperature-treated material there may be found among normal pollen mother cells which are derived from affected premeiotic tissue a few that are tetraploid. The number of these cells was estimated at not more than three per cent. In studying premeiotic tissue in very young anthers, significantly enough we find a very small percentage of cells at metaphase stage, while the great bulk of cells are in other stages of nuclear development. It seems apparent that the temperature changes may have affected only the somatic chromosomes at the metaphase stage, as presumably occurs in the meiotic phase; therefore, only chromosomes at this stage may be forced into clumping and into the resting stage, and consequently from such treatments the percentage of tetraploid cells may never be high. Perhaps this may account for the fact that a higher percentage of polyploid plants has never been obtained through somatic doubling at the first stages of zygotic development into embryos of plants or elsewhere by means such as temperature, X-ray, or centrifuging because their effects are temporary and not extended. To produce more polyploids by the above methods, such treatments may have to be repeated at certain intervals in order to obtain the desired result.

In contrast to somatic tissues, during various meiotic phases most of the pollen mother cells are at the same stages. Thus, when chromosomes are forced into the resting stage, at either reductional or homotypic metaphase stages; or as a result of univalency brought about by affecting the leptotene stage, all the pollen mother cells in anthers for a period of time may be affected and produce diploid microspores instead of regular haploids. It seems, therefore, that the effect of temperature is primarily on the chromosomes, which seem to react to temperature changes by contracting or clumping, and secondarily on whatever mechanism is involved in chromosome separation or cell division, by a change in the viscosity of the cytoplasm. This change in viscosity or any other disturbance in the cytoplasm in turn may prevent the formation of the spindle mechanism, or may affect physicochemical forces that may be instrumental in the separation and movement of chromosomes toward two poles.

As we saw above, the change in temperature from optimum to either high or low can be made quite slowly and polyploid cells may still be obtained in *Rhoeo discolor*. In the case of the cold treatment, for example, the change from a 15° C. temperature to 4° C., was brought about in five hours' time. After keeping the plants in 4° C. atmosphere for 18 hours, the air in the cold box was allowed to rise to 16° C. during an interval of 12 hours. The result here indicated might suggest that, at least for *R. discolor*, the change of temperature to effect polyploidy does not have to be made

abruptly; and that the fall in temperature from normal to an effective low degree in a five-hour period was rapid enough for this material. However, for some other plants it is probable that the change from an optimum to an effective temperature for induction of polyploidy may have to occur quite suddenly. If such a difference in response in different plants occurs, it may depend on the rapidity of chromosome division and also on how quickly the cytoplasm of a dividing cell reacts to such a temperature change taking place in nature.

Polyploidy by colchicine treatment.—The end results produced by any method appear to be the same, namely, the number of chromosome sets is increased by multiples of two. But the way any particular treatment brings about such a doubling of chromosomes may vary. Colchicine, as we will see, outwardly produces the same effect as extremes of temperature, but in its mechanism seems to differ considerably. One major difference is that the colchicine effect seems to be more *extensive*. As we have seen, the effect of a temperature change appears to be almost immediate and only those cells may be affected in which chromosomes were either at metaphase stages—reductional or homotypic in meiosis and mitotic in somatic tissues — or at the leptotene stage. In either case, temperature changes may affect the chromosomes and in various degrees may clump them together perhaps during all phases of nuclear development, and also may bring about a temporary disturbance in and arrest of the mechanism of nuclear and cell division.

With colchicine the chromosomes at no time appear to be affected. The fragment referred to in Figure 5*A* may be due to an entirely independent factor, since in no other instance were fragmentation and abnormalities of similar character encountered in colchicine-treated material. The drug seems to upset only the mechanism involved in nuclear and cellular division. This appears evident from the fact that in an affected cell the chromosome multiplication may occur more than once, while in temperature-treated material this never takes place more than once unless, perhaps, by repeated treatments. In colchicine-treated cells the sister chromosomes, as a result of mitotic splitting, remain together instead of being moved to opposite poles, and this may go on for some time as long as colchicine is present in sufficient quantity to be effective. Colchicine, then, is an internal agent and will act as long as it is present in an effective concentration within the cells, while temperature is external and will be effective immediately and only at the time of application. With colchicine treatment the cell volume and cellular wall are increased, apparently in proportion to chromatin increase; therefore, actually there is no stopping of protoplasmic growth by the drug, and along with chromatin increase there seems to be an increase in the cytoplasm as well.

By neither method could the multiplication of chromosomes in meiosis be extended further than what seems to be predetermined by an inherent factor. As a result of normal meiosis, microspores contain the haploid number of chromosomes. The sum total of chromosomes in four microspores produced from a pollen mother cell is twice the diploid number. When polyploid microspores were produced by the above methods, microspores were never produced which contained more than twice the diploid number of the pollen mother cells. This inherent factor may perhaps control the chromosome number in male gametes as well; therefore, we may never obtain male gametes containing more chromosomes than their parent microspore. The author only once found a case where the total number of chromosomes produced from a pollen mother cell was more than twice the diploid number (original observation from a plum and apricot hybrid). In the same pollen mother cell there were eight microspores (octet) instead of four (tetrad), and unquestionably the individual microspores were the same size

as the normal ones. Here, then, after a reduction division, homotypic division had occurred twice.

The extent of multiplication of chromosomes and resulting abnormalities in somatic tissues is apparently dependent upon the concentration of the colchicine solution applied. In one experiment, 0.1 per cent, 0.05 per cent, and 0.025 per cent concentrations were applied on Rhoeo flower buds. With the lowest concentrations no effect was observed on stamen hairs, and occasionally there was found some effect by the higher concentrations. It seems, therefore, that by finding the right concentration it may be possible partially to control the rate of chromosome multiplication in a given species or variety of plant. The disturbance accompanying the application of higher concentrations seems to bring about a distorted growth in the treated material because not all cells are uniformly affected. Some cells may be active and chromosome multiplication and increase in cell volume as a whole may continue some time, while other cells may not be so active, or some may have ceased to develop and become differentiated. From such a state of affairs the whole affected tissue may suffer and eventually die.

Specificity of temperature and colchicine effects.—It is apparent that extremes of temperature result in clumping and probably shortening of chromosomes. This is evidenced from fusion and fragmentation of chromosomes following such treatment to be discussed below, and from the behavior of chromosomes during reductional division, which had been affected at leptotene stage. But with either method, both nuclear and cellular divisions may be prevented and chromosome doubling may be brought about. Although the results from either method may be the same, the factors responsible for bringing about such results may be different. If a difference in the factors effective in these two methods exists, it may be apparent from the behavior of chromosomes at the leptotene stage. With either method, leptotene chromosomes fail to pair and they remain univalent. With the temperature effect as we have stated, all the chromosomes are similarly affected and leptotene asynapsis may be entirely due to shortened chromosomes. With colchicine, however, such a phenomenon is not apparent, since chromosomes may multiply many times, indicating that chromosomes do elongate through the resting stages and are thus self-perpetuating. When chromosome elongation at leptotene stage is not at a maximum chromosomes may also fail to pair. However, as long as enough colchicine is present, nuclear as well as cellular division and synapsis of leptotene chromosomes fail. Whenever there is an asynapsis of leptotene chromosomes, there always appears to be a failure of first meiotic division. It was observed in the temperature-treated material that when the pairing of meiotic chromosomes was partial, and some were paired and others not paired, the chromosomes in the pairs appeared to be polarized while the others remained, one might say, neutral. It may therefore be suggested that colchicine has a direct neutralizing effect on the force or forces of the division mechanism, while the effect of temperature change on the division mechanism is indirect. The latter may have resulted from the action on the chromosomes, and the former from the action on the cytoplasm which may be considered the source of the nuclear and cell division mechanism. Thus, it may be suggested that temperature effect may be more specific on chromosomes, while colchicine effect may be more specific on cytoplasm.

Fragmentation and fusion — In March, 1936, in the temperature-treated material of Rhoeo, fragmentation and fusion were observed at the metaphase of the first nuclear division, mostly in diploid microspores and occasionally in haploids. The experiment which had resulted in fragmentation and fusion was repeated toward the end of the same year. At this time,

the process of gradual cold and heat treatments was also applied. When treated material was scrutinized, fragmentation and fusion were apparent in the material subjected to all manner of temperature treatments. Judging from the time of appearance of these abnormalities, it was obvious that temperature treatments were effective on all stages of chromosome development and the effect was on both somatic and meiotic chromosomes.

Barring other factors which may bring about fusion and fragmentation in chromosomes, clumping brought about in Rhoeo by temperature treatment seems to be the factor effective in inducing fragmentation and fusion. This is deduced from the fact that no such phenomena were observed in the material subjected to colchicine treatment, since by this method no clumping was caused.

It is interesting to note that fragmentation and fusion of chromosomes are usually observed in material treated with X-ray and by similar methods. In these phenomena, if the underlying factors are not homologous, then their analogy may be worth recording here.

Suggestions for Inducing Polyploidy

The following suggestions may be helpful in polyploidy experiments with colchicine. An accurate knowledge of the stages of meiotic phases of flower buds and the rate of activity of meristematic parts or young seedlings treated is vital for successful results. No effect may be expected from treating vegetative parts that are not growing vigorously. It is unlikely that colchicine will affect cells that are not active, since (as is indicated in the text), the colchicine solution is an agent that, by being diffused into active cells, disturbs the cell-division mechanism. Inactive or dormant cells may be affected only if they become active while colchicine is still present in them in sufficient amount to affect the cell-division mechanism.

Different plants may require different concentrations of the chemical for the most effective result. Solutions of colchicine as low as 0.025 per cent and as high as 2 per cent have been used experimentally. However, it is believed that most plants will yield results with the application of a 0.2 per cent solution. It is advisable to divide up the samples of the material to be treated into groups and apply to each sample a different concentration of solution. The length of treatment may also be varied, using from 1 to 16 hours. The method of application may be by wetting, immersing, or soaking, depending upon the material to be treated. The part to be treated may be wetted either with the aid of a camel's-hair brush or by placing on these parts a small amount of absorbent cotton thoroughly saturated with the solution.

The suggestions as to the treatment of different types of material are as follows:

1. Producing diploid or higher polyploid pollen grains and possibly polyploid eggs.—Wet with or immerse in colchicine solution young flower buds before or after meiotic chromosome pairing, or at the time of meiotic metaphase. Duration of immersion may be one hour or longer. This method is to produce polyploid gametes and by their union polyploid embryos. The question whether polyploid pollen grains produced will be functional or not may be determined only after trials, since in some species of plants polyploid pollen grains have not been functional on diploid individuals, while in others such a combination has succeeded.

2. Inducing polyploidy by affecting embryos at the very beginning of their development.—In plants like strawberry (Fragaria), blackberry (Rubus), Petunia, tobacco (Nicotiana), and in many other plants where the embryo development is not deep-seated, young fruits may be treated by thoroughly wetting with colchicine or immersing in a solution for one hour or longer at the beginning of embryo development. Before embryos are initiated, the treatment may prevent embryo de-

velopment entirely. The time of embryo development may be days (5 to 6 days in strawberries)*; or weeks (about three weeks in grapes and peaches),* and at times, a year (the family of pines), after pollination. There often seems to be a break between the time of fertilization and actual embryo development, which should be determined to obtain satisfactory results.

3. Inducing polyploidy in very young seedlings.—Seeds may be germinated on filter paper or elsewhere. One- or two-day-old seedlings may be soaked in solution for three or more hours (depending on the size of seedlings), planted in pots, and covered lightly with soil. Young seedlings already in soil, if they can be handled conveniently, may be uprooted, washed, and treated as above. Seeds of strawberry were treated and no results were obtained (judging from the external appearance of the young plants), while the treatment of the seedlings reacted favorably. We now have from this source a number of plants of *Fragaria vesca semperflorens,* that pollen grain examination indicates are polyploids. The difference in the reaction between seeds and seedlings seems to be due to the fact that the latter are actively growing, hence they react to colchicine treatment, while the former are dormant at the time of treatment. The success of seed treatment in the cases reported by Blakeslee and Avery seems to be due to the fact that the seeds treated by them may normally germinate quite soon after the treatment, while in the strawberry it requires about a week to germinate, by which time the colchicine in the seeds planted in the soil may be so diluted—if not diffused out entirely—that it is not present in sufficient quantity to have any effect on the mechanism of cell division in the dividing cells of germinating seeds.

Distorted growth often witnessed in the colchicine-treated young seedlings may be due to a prolonged treatment. Such an effect may possibly be prevented if seedlings are exposed to the drug for a shorter period, then washed in water for a short time to stop further effect of the colchicine.

4. Inducing polyploidy in growing tips.—Growing tips are either wetted with or immersed in the solution for three to sixteen hours. Rapidly growing plants will react to the treatment more favorably than slow growing ones. If tomentose tips of plants are wetted with 0.1 per cent Aresket, the colchicine solution will penetrate between these hairs and reach the epidermis and diffuse through it more effectively.

The author was able to produce tetraploid cells in root tips of apple, cherry, lima bean, peach, and sweet corn by placing young seedlings in ice water at a temperature of 1° to 3° C. for two hours. Similar results were obtained by placing very young seedlings in hot water at 35° C. for one hour. Much shorter time and a little higher temperature for hot-water treatment may give better results, while with ice water the time may be extended without injury to the seedlings. Heat treatments were given to the young seedlings of apple, cherry, and peach.

High percentage of polyploid pollen grains may be more successfully produced by treating young flower buds, at proper stages of pollen mother cell development, with low or high temperature than with colchicine. Diffusion of colchine solution into the anthers may not be rapid and even, while the temperature effect is, comparatively speaking, immediate and even.

Tetraploidy or higher polyploidy of resulting plants may be first detected by comparing stomata of leaves of normal and affected plants, or by the size difference of pollen grains produced. The affected parts are either propagated by cuttings or by seeds that may be obtained by selfing the flowers. The type of polyploidy may be accurately determined by the chromosome

*From original observations not yet published.

counts from root tips of cuttings and seedlings.

Summary

1. The methods of inducing polyploidy in the meiotic and somatic cells of *Rhoeo discolor* with colchicine and temperature changes are described.

2. Gross and cytological observations are presented concerning somatic changes brought about by colchicine treatment of the Rhoeo flower parts. It was found that colchicine enters tissues and inhibits cell division, while the nucleus and cell volume may increase, presumably, as long as colchicine is present in sufficient quantity to be effective, until such an increase is checked by some other factors.

3. Colchicine is effective on both premeiotic and meiotic cells. Pollen mother cells derived from affected premeiotic tissue may be $4n$, $8n$, or higher, depending upon how young the treated buds were at the time colchicine was applied and how many times division in the premeiotic cells has previously failed before being differentiated into meiotic cells. By affecting normal pollen mother cells, either diploid pollen grains are produced if the effect of colchicine is after reductional division or tetraploid pollen grains if the effect is before reductional division. The treatment was apparently ineffective in causing chromosome doubling in microspores.

4. The immediate result of temperature treatments is the same as that of colchicine. However, it is shown that the effect of temperature changes is immediate and not extended, as is usually the case with colchicine. Here lies the most important difference in the effects of these two methods. It is suggested that temperature effect may be more specific on chromosomes, while colchicine effect may be more specific on cytoplasm.

5. Evidence is presented to account for the fact that by temperature-treatment the percentage of polyploid seeds in heat-treated material is small, and an attempt is made to account for the rarity of polyploidy occurring in nature.

6. In temperature-treated material, fragmentation, fusion, and chromatin bridging occur. It is suggested that clumping brought about by temperature treatment is responsible for these features.

7. Some suggestions are presented for plant breeders who are interested in doing experiments in polyploidy.

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TOWARDS CURING DIFFERENTIAL BIRTHS AND LOWERING TAXES

A Plank or Two for Eugenic Platforms

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SOME months ago the Editor of the JOURNAL expressed the need for constructive planks for eugenic platforms. Some race improvement timber has been put forward for the inspection of the JOURNAL audience, but not a great deal. However, there is one absolutely sound, strong plank among the lot, which just now it is high time to sieze and thrust into the fray. As additional millions of unemployed crash down upon Relief every group and profession of Americans must rally to meet the resulting economic crisis. Whatever our political, economic, or theological outlook is we are at one in admitting that this crisis exists. Now some of us who read this JOURNAL are wasting our time—we are trying either to calculate the uncertain millions of babies born on relief since 1929, or throwing up an emotional smoke screen in a counter argument that few of such babies are different enough from average babies to give such calculations any value.

But neither of these reactions meets the crisis. Just as lawyers simply must get busy about monopolies, and bankers about the credit situation, and other professions about their specialties, so eugenists must do their bit today on what they know about—not much, but their bit. Among our 30 million dependents there are some—maybe one million, maybe ten, against whose continuation it is time to move. It is our responsibility to cut off a bit of the degenerate tail-end of this menacing army, whether the degenerated tail turns out to be a furlong of the army or a mile of it, and even if we can only clip a yard off for the present. To do this we must all inform ourselves (A) of the permanence of the present evil tax situation, (B) of the practical steps available about which we all basicly agree, whether we be Catholic or Protestant, Economic Royalist or New Deal.

A—The Burden

Good unpublished estimates from the University of Pennsylvania and from the committee in charge of the statistics for the American Conference on Social Work, assure us of this: there are 15 million people on the public purse in good times, 30 million in the worst times.[1] These figures are both of them *minimum* estimates which include the burden on local philanthropists and taxpayers but take no account of all that is also done for indigent personal friends and kin.

Since there have never been 30 million solvent households in this country at any time and in recent years, very much fewer, it works out that each home has, on an average, been supporting by taxes and quite impersonal charity about one person outside its own family. In the hard times that we have known since 1929 there have been about one and a quarter—and possibly as much as two—of these unseen guests at each family board; perhaps sinking in the best boom times as low as three-fifths of a person uninvited in your home. *But usually there is an unseen guest at every table*—besides (perhaps) Grandma who is often either sitting there in the too, too solid flesh or else is on subsidy somewhere in her own apartment,—an additional burden on the harassed breadwinner.

We may well ask ourselves for what end the wheels of American business and industry spin so dizzily. Is it for

the support of happy, self-respecting children who will have opportunity to become useful citizens, or is it to keep—or rather, alas! only half-keep—people on relief and their babies? There is something radically wrong with our management when this unwanted guest sits down at every family dinner eaten by parents with children; a specter, yet one which must be fed, clothed and lodged; a pitiful and difficult being hovering somewhere just out of your sight, always an object of public or philanthropic expense, and furthermore, too often a blot upon the nation's fair page, because he has the further misfortune to be insane or feeble-minded or criminal.

Only 59 per cent of all our 30 million homes, solvent or insolvent, have one child or more residing in them in any given year.[2] So you see, there are even in boom times about as many dependents and parasites as there are happy, solvent homes with young life growing up in them. Per home: 1 or 2 parasites, 1¼ children.

In hard times, public spending goes on at the rate of $530 per family[3] and more in the higher brackets. It is, then, no wonder that the income-tax-payer averages (married or single) something more than one child to grow up and be fertile[4], and the man on the dole more than two.[5]

Something is very wrong indeed when in nine leading states the native-whites, both high and low together, are dying out at the rate of 10 to 30 per cent per generation.[4] One surmises, therefore, that many "high-born" groups may be declining by 50 per cent. Further, Dr. Frank Lorimer states and Dr. Notestein is in agreement, that while areas of vast importance are scarcely reproducing their populations, the "agricultural problem areas" have 76 per cent more babies than needed for replacement.

B—The Steps to Take

Now, there is more than one way of curbing helter-skelter fecundity by the riff-raff. In certain past ages, it was often more or less effectively done—by one or another method of reserving all females as servants or mates for the solid substantial citizens only.

Your attention is invited to one possible modern method, already foreshadowed in South Dakota, and very hopeful.[6]. Dr. F. V. Wilhite reported last summer to the American Association on Mental Deficiency[7] that South Dakota has succeeded, in only five short years, in getting control of more than one-quarter of all its mental deficients, this quarter covering *all 12 to 30 years old* and many of other ages. Like most states it has only one-tenth of its feeble-minded in institutions.[8] But unlike most states, it takes a careful continuous census of the other nine-tenths too. All these (5,762 known feeble-minded) it will eventually register and control as wards of the state, some in institutions, but far more outside of them.

The list of between one and two thousand mental deficients at large in the various counties (but under supervision of the State Commission and county sub-commissions as legal wards of the state) has been sent to every marriage clerk in the state. Each clerk is under severe penalty not to issue a license to any one on the list (except where the State Commission has approved their ward's sterilization and had it carried out). Where a ward steps out of the state to get married—or shows signs of becoming promiscuous without marriage, sterilization becomes mandatory. So far, 248 operations have been performed, 61 per cent with the consent of relative or guardian.[7] In Catholic cases the state ought rather, perhaps, to order segregation under the bishop's control with state funds provided to cover the expense.

In states where there is any sentiment against sterilization—and that includes many of the twenty-eight states where compulsory sterilization is now legal but a dead letter—it will be feasible to depend mostly on segregation, with voluntary sterilization used merely as an avenue of release to such as wish to avail themselves of it.

The practical road towards dealing with all the permanently Unfit and Unadjusted must follow the South Dakota

program of registration and control, captained by Dr. F. V. Willhite. And, whether sterilization is used or not—this is not the chief point of interest. Also, whether a program of government control is dealing with the feeble-minded's birth rate or with the birth rate of quite other classes of the Unadjusted, neither is this the most important thing. The point is, to establish legally that government has the right to declare any permanently unadjusted person (who has burdened the taxpayers for years) to be its ward, and to handle him or her as is necessary to limit parenthood or prevent it entirely, though controlling the *hers* is all that is necessary to gain much of our object. Those Unadjusted who are not feeble-minded may (just possibly) need a very different program from that of South Dakota, but the way to discover this different program is, we firmly believe, along the South Dakota road. *Proper handling of the feeble-minded—who are a great part of the problem-people, anyhow—will indicate whatever slightly different program will be practicable for the rest* of the Unfit and Unadjusted—the remainder of those who are a continual drain on the tax-payers, who never pull their weight in the boat, because of some combination of psychopathic, physical, or "dull mental" faults. Competent judges think that far more than half of the Unadjusted are suffering from much the same mental inadequacy—though in a less degree—as the definitely weak-minded. One per cent of any large population is "feeble-minded"; six per cent can not finish the elementary grades.[9] Practice by officials in programs to keep this one per cent childless, or nearly so, will teach us how to limit the six per cent and also all other permanent Problem-people to a replacement birth rate only. Until a family or a group has proved its ability to attend to its own, can it claim *more* than replacement?

Identification and registration of the feeble-minded has been a prime object of the American Association for Mental Deficiency for twenty years. Selective sterilization has been an objective only since 1929.

The earlier effort is more in the line of solid progress. But the chatter about sterilization, however loose, has been a valuable symptom that society means to come to grips with the problem. So now without a long wait to settle the quarrel over the righteousness of either sterilization or birth control, let us appoint guardians to these people, pay each guardian to make careful annual reports on his charge's sex and family relations, even if he can not actually at present regulate them to any great extent, and in time a technique of regulation will develop itself.

Social case work and judicial procedure have already on hand a whole pharmacopeia of remedies for indiscriminate breeding. But for lack of laws like that of South Dakota, the remedies cannot be applied. Let us run over a list of these remedies, beginning with those acceptable to Roman Catholic opinion.

Here is a mother of twelve children hating the sight of the idle brutal father and desiring above all never to set eyes on him again. The whole family has had charity for years. Before she has a thirteenth child, a separation (not divorce) should be arranged, after he or she has been certified by the Courts as a permanently unadjusted person not very likely to provide a proper home for even three children on a real American standard. Desertion has been an immemorial means by which a husband gets rid of a too fertile wife. Wives would even more frequently adopt separation from too fertile husbands if this did not involve loss of support and Church condemnation. It is probable that the churches will eventually become more lenient in countenancing separations from too-fertile non-providers. The question of divorce does not enter seriously here, because the man can not provide for any other woman any better than for this one, and the wife will be as incapable or as unwilling to use contraceptives with another husband as with this one. What the mother of twelve

children often wants most is an assured income rather than any more sex.

Now suppose the woman of the preceding case has been given her judicial separation, with allotment of a fraction of the man's scanty casual earnings, to be supplemented by public relief such as the family has had for years, anyhow. But she now begins to consort with other men. She thus becomes a candidate for imprisonment as a loose woman. We submit that somebody else should be dismissed from some institution of the state—some mildly insane person, some minor criminal—and that she be incarcerated in the place thus vacated. (This is suggested so that the whole eugenic program be not held up by taxpayers' anger at having to pay for *more* institutions.) Better that we have all the blind begging on our streets again than that such as she be at large. It is such as she who are the chief fountain source of blind beggars. But if absolutely no existing place can be found for her in an institution, let some vacant building be opened for the temporary detention of all such as she, at a very minimum of expense. If the community remains opposed equally to new institutions or to the voluntary sterilization of these women so that they can be released from their temporary quarters with safety to the community, it will then be time enough to resort to the Japanese factory system described below, or to some other device.

But there are other cases and types of solutions of this whole problem. Take a mother of two, of "dull normal" mentality, with no playground for the two she has except the gutter, with husband in and out of court occasionally for various delinquencies and misdemeanors. The family is "unadjusted," that is to say, they are on continual subsidy from the tax-payers or private philanthropists. At present the possibilities of birth control have not been fully utilized for such as she. Suppose she is a Roman Catholic. What is to prevent her being declared a ward of the State, and paroled in the care of the bishop of her diocese, together with the charitable funds which she has been accustomed to receiving? Using part of these funds, the bishop could set up for her and others of his flock who had been similarly paroled a special medical supervision—a "rythm" type of birth control acceptable to Catholic doctrine.[10]

The needed continence could be induced by having the women and children live in a Catholic institution of the cottage type, with the husband permitted to visit during only two-thirds of the month. Or the families could be left at large in the community and each husband, as a condition to receiving a certain amount of relief money, required to "go into retreat," boarding at night at a Catholic hostel for men only, during a small part of each month. It is not supposed that such a system would prevent all births, but it would cut down the rate enormously, which is all we can expect at first, and until better methods are developed. Even to space out the children by a few more months would be worth while for the sake of improving their health and morale.

If such systems seem impossible, it will be so only to readers who have not studied the marvelous success with which taboos, such as the ritual laws of the Old Testament among the Jews, can be enforced upon people in every part of the world by their tribal and religious leaders. If the Catholic Church is not at present able to bring the benefits of modern hygienic discipline to its people, it may be because it no longer has much support from organized government. Church and State working together to discipline the undisciplined and unadjusted might go far.

Where Unfit home-makers cannot or do not limit their children to the number they can properly bring up, a government Registrar or Commission may soon make choice among the following remedies:

Reduction of fertility by glandular or serum treatment (not quite yet perfected).
Birth Control (Catholic type, or otherwise).
Separation (not divorce).
Voluntary sterilization.
Compulsory segregation in an institution.

This last—the ultimate recourse when

all else fails—has a most peculiar status. At first glance, it seems very expensive per capita and perhaps unendurably distressing to the person shut up. But in practice, the total expense and total cruelty might be small. In the first place, this ultimate weapon in the government's program could be used far more as a threat than as an actuality. Because people care more for their liberty than for almost anything else in life, even a temporary taste of confinement is a powerful dose. By making an example of judiciously selected cases here and there, imprisonment might at small expense (because of being temporary) and with small cruelty (for reasons to be described) have a vast effect in inducing a wider use of birth control and thus reducing the birth rate where it needs to be reduced.

Even permanent custody can be made less cruel when done for eugenic ends than when done for any other purpose—for the reason that the women might be given their liberty to go home for a few days or a week out of every month.

The South Dakota law, modified to satisfy Catholic opinion, should be passed by the next legislature in every state.

(To Be Concluded)

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1. Unemployed *with dependents*, 7½ million; unemployable ditto, 4½ million; institution cases plus unconfined criminal parasites, 3¼ million. This 15 million becomes 30 million at the nadir by the tripling of the unemployed which occurs. See Table A-2, p. 68, H. L. Hopkins to the Sub-committee of the Com. on Approp. of the House of Rep. on Deficiency Appropriations, 75th Congress, 1-13-37. P. 160 suggests 2½ million are the normal unemployed. To include "dependents" too, statisticians usually multiply by 3. P. 92 gives "unemployable."

2. *U. S. Statistical Abstract*, '37. Table 42.

3. Dennett, Carl P. National Economy League (Phila.) pamphlet, "Soaking the Poor." Public spending exceeds taxes.

4. Lorimer and Osborn. Dynamics of Population, N. Y. 1934. p. 11, p. 358. We estimate from p. 18, by supposing the income taxpayer has a birth rate similar to the business class in general, that is, 15% below the general rate of his locality—the usual situation in most places studied. Thus, reducing the rates of Table 2 by 15%, we find: in 4 great "divisions" of the U. S. where the most tax is paid the business man would raise from 1.3 to 1.7 fertile child apiece. Lorimer, *Birth Control Rev.*, April, 1938, p. 79, suggests city rates yet lower.

5. The people that go on the dole are from classes having a net reproduction at least 10% above the nation in general (which is at unity). This is got by applying Lorimer and Osborn's occupational reproduction indices, p. 74, to Hopkins' Table 37 (Ref. 1, above), and would be greater yet if allowance could be made for the fact that the part of each class appearing on the dole in a given year is the part that happens to be burdened at least that year with far more children than the rest of its class, according to Sydenstricker, to Stouffer and to Melvin's "Rural Youth on Relief" [a 1937 Monograph by W.P.A. showing (p. 70) 34% more people under age 15 in the rural relief population than in the general rural population].

6. Journal of Heredity. Oct., '36. J. H. Craft.

7. 1937 Proceedings of 61st Session (Vol. XLII, No. 1 of the *Jour. of Psycho-Asthenics*), Elwyn, Pa. p. 97. S. D. 6th Biennial Rep., State Comm. for the Control of the Feeble-minded, June, 1936.

8. White House Conf., Com. on Physically and Mentally Handicapped (Wm. J. Ellis) "The Handicapped Child," N. Y. 1933, p. 4, p. 334-46.

9. Huntington, Ellsworth. Tomorrow's Children, N. Y. 1935, p. 43 suggests that, though some of the 13% declared mentally deficient in Ref. 8 may be intellectually able to finish sixth grade, an additional per cent does not, that is, can not do it for other reasons. So, to say 6% "can not" finish, is a very moderate estimate.

10. The Pope's 1931 Encyclical. Also, O'Brien, Rev. John A. (Catholic Chaplain of the Univ. of Illinois). Legitimate Birth Control (1934), authorized by the Bishop of Fort Wayne.

IVORY, A FERAL MUTATION IN PEROMYSCUS*

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THE breeding of trapped specimens of *Peromyscus maniculatus*, taken from widely separated locations in North America, together with the breeding of their immediate descendants born in captivity has produced a fair number of mutant types. Since the actual number of wild parent mice, whose genotypes have been tested, is not large, it may be inferred that recessive genes are widely distributed in wild populations and carriers of these are fairly common.

In spite of this the taking of an easily identifiable mutant specimen in the field is an uncommon event. Osgood[4] examined 27,000 study skins in his revision of the genus and mentions but three noticeably aberrent specimens. Since study skins can be put up in a short time and anything out of the ordinary appeals to a collector it seems probable that this dearth of mutants is due to their rarity. More recently many thousand mice have been taken in live traps by Sumner, Dice and others on the lookout for mutants with equally meagre results.

One might expect this to be due both to the relatively rapid elimination of mutant types in the normal environment of the species and to behavior which would tend to reduce inbreeding. With regard to the latter possibility, which geneticists would presumably be less familiar with than the former, it has been ascertained by animal ecologists that domestic arrangements actually are such that young mice upon graduation from the home must, as a rule, journey considerably afield before settling in permanent homesites of their own.

The circumstances attending the taking of the ivory mouse to be described are so preposterous that they are worth recording for the benefit of those interested in the anecdotal fringe of science and perhaps as a flagrant case in point for practical minds who would inveigh against the sizing of scientific articles with the filler of irrelevant detail. I had received a request for some wild Peromyscus from Dr. A. Packinian, now of the National Institute of Health, who was interested in testing wild specimens of the genus for trypanosomes. The election for the job was a thrice removed one since it had come by way of the University of Michigan and the Scripps Institution. After agreeing to supply the mice I had, with characteristic larceny of time, allowed the matter to rest until a letter of reminder aided a nagging conscience in prompting a trapping trip to a location near Eugene, Oregon, which had in the past yielded considerable numbers of deer mice.

In this site a one-time formidable tangle of evergreen raspberry bushes had lately bowed to agricultural progress and the locale, once a cover for the activities of Melospiza by day and Peromyscus by night, had been largely incorporated into an orchard, the cover along the rim of which barely accommodated a battery of ten traps. After considerable search for more trapping terrain proved fruitless one more trap was set a little removed from the ten. This most forlorn, of a group set unhopefully, took the original ivory specimen: a male in adolescent pelage, together with the normal *P.m. rubidus* of approximatley the same age pictured with him in Figure *7A*. To

* This research was supported by the Research Council of the State System of Higher Education and the National Research Council.

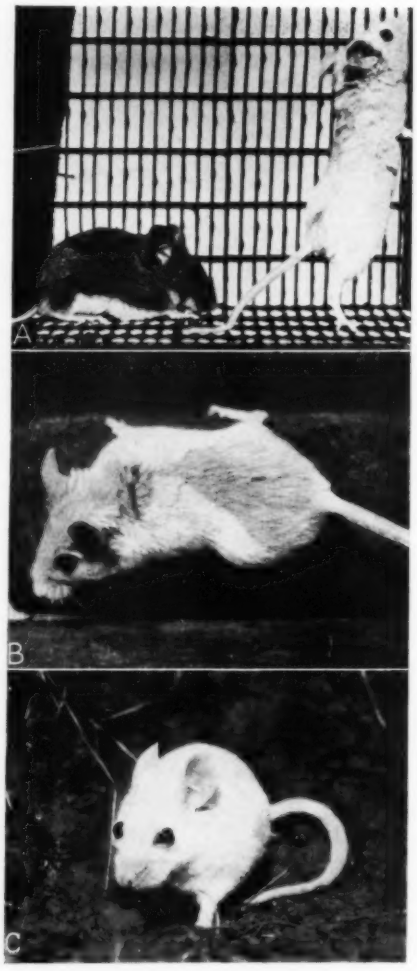
complete the story, one of a number of mice taken in the other traps rewarded Dr. Packinian's search for trypanosomiasis in Peromyscus.

Description of the Character

As may be corroborated by attention to Figure *7C*, an ivory deer mouse in adult plelage, the adult coat is, on the surface, almost as light as that of an albino. When compared side by side with albino Peromyscus—which appear, incidentally, "whiter" than albino Mus—ivory is enough darker to be separated from albinism at a glance and a confirmatory test can be made by parting the hair on the pelage. The basal portions of the ivory hairs are darker than their own tips and substantially darker than the basal portions of albino hairs. The eyes of ivory mice are red and appear darker than the eyes of albinos.

There is considerable effect of age in the ivory coat. Juvenile mice are whitish brown, this coat being in most of the specimens, observed so far, considerably different from the adult pelage and quite dark enough to show the position and extent of white spotting if this is present. This brownish coat is gradually replaced, after five or six weeks of age, with an adolescent coat much nearer the adult color. A mouse with juvenile fur on head and rump separated by an encroaching saddle of whiter post-juvenile or adolescent hairs is pictured in Figure *7B*. There is some variability in shade, this mouse appearing a little darker than average in both cases.

In normally colored specimens of Peromyscus the basal portions of all pigmented hairs bear linearly arranged clumps of black pigment granules in medullary lacunae[2]. The distal portions of hairs of the ventral surface are colorless while the distal portions of dorsal hairs either continue the black medullary pigment close to the extreme tip, where it is supplemented with corticular brown or black pigment, or interrupt the black medullary pigment with a subterminal agouti band of yellow. In ivory hairs neither subterminal yellow pigment nor distal corticular pigment can be seen under the microscope and these portions of the hairs look white. The medullary clumps of black granules are represented



IVORY AND NORMAL
Figure 7

A—Standing: the original ivory mutant. Seated: normal *P.m. rubidus* males taken in the same trap. *B*—Young ivory mouse showing pale brown juvenile coat on head and rump and saddle of whiter incoming adolescent pelage. *C*—Adult ivory Peromyscus. The red eyes photograph black in all pictures.

by small residual masses of brownish looking debris which occasionally include an almost complete dark granule. The white hair tips and white subterminal bands account for the pallor of the surface of an adult ivory pelage in contrast to the darker color of the proximal portions of the hairs, all slightly pigmented in their medullary portions. Older mice appear to be paler because smaller amounts of the brownish medullary pigment are deposited and probably because of some difference in the dimensions of the white portions of the hairs.

Ivory mice differ from pink eye in the lack of yellow pigment, the reduction of black pigment being more or less equivalent in amount in the two coat colors so that the basal portion of the pelage in the two mutant types is similar in appearance[1,5].

Young silver agouti mice may be as pale as ivory specimens in first coat but "silver" hairs have slightly pigmented tips and white basal portions while ivory hairs reverse this arrangement. With age silver agouti pelages get darker and ivory pelages lighter, while, in addition, silver agouti mice have black eyes[3].

If ivory had appeared in a pale Peromyscus subspecies, such as *P.m. sonoriensis,* instead of the very dark *P.m. rubidus,* the adult ivory coat would have been almost indistinguishable from albinism. In *P.m. sonoriensis* ninety-five per cent of the dorsal pelage hairs are banded and as these agouti bands are wide, an ivory sonoriensis pelage surface would look about ninety-five per cent white.

Inheritance

Ivory mice have been mated with the following other coat colors: albino, pink eye, brown and silver agouti. In all cases these matings have produced wild type young only. Matings with brown mice have been most numerous and enough young have been reared to indicate that ivory coat color is probably due to a recessive gene substitution. The data are tabulated at the right.

While many mutants in Peromyscus parallel characters already known in Mus ivory does not appear to do so. It may possibly bear some genetic relationship to one or other of the almost white coat colors associated with reduced eye pigment in cattle, dogs and guinea pigs.

Summary

1. A young ivory male *Peromyscus maniculatus rubidus* was trapped at Eugene, Oregon. It had an almost white pelage and red eyes.

2. Ivory mice are pale brown in juvenile pelage, become whiter in succeeding pelages.

3. The dark cortical pigment and the yellow pigment of the agouti bands, present in normal pelages is not deposited in ivory hairs and the normally occurring medullary groups of black pigment granules are reduced to masses of small brownish particles.

4. Genetic test shows ivory to be due to a recessive gene substitution not allelemorphic with albino, pink eye, silver agouti or brown.

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Table 1

Mating	Offspring		
	Wild type	Brown	Ivory
brown × ivory	240	—	—
exp. 1 : 0 : 0	240	—	—
b × *i* F_1 × ivory	124	—	130
exp. 1 : 0 : 1	127	—	127
b × *i* F_1 × F_1	148	49	52
exp. 9 : 3 : 4	140	47	62

LEAFLETS REDUCED TO ONE

Figure 8

A—Unifoliolate *Trifolium repens* clonally isolated from one such plant found in an open-pollinated population of 10,000 individuals (× ½). *B-F*—Representative types of leaves from the unifoliolate plant showing leaves with two or three leaflets as well as intermediate types (×2).

A "ONE-LEAVED" WHITE CLOVER

Unifoliolate Mutation Appears Among 10,000 Plants

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U. S. Regional Pasture Research Laboratory, State College, Pa.

THE white clover (*Trifolium repens* L.) nursery at the Regional Pasture Research Laboratory consisted this past summer of nearly 10,000 spaced plants. They were started from seed in the greenhouses during January, 1937, transferred to pots in February, and set out in the field during May. When the population was examined carefully at the end of the summer, one plant was found which had leaves with single leaflets instead of the usual three. It occurred among a group of 200 plants grown from open-pollinated seed collected in an old pasture in West Virginia.

Figure 8*A*† shows the growth resulting from three small slips of the original plant after four months in the greenhouse. Practically all leaves are clearly unifoliolate (Figure 8*A*). Occasional leaves may have more than one leaflet; this is especially true of leaves borne on the relatively short axillary branches. Both bifoliolate (Figure 8*E*) and trifoliolate (Figure 8*G*) leaves are found; intermediate conditions with irregularly shaped leaflets occur less frequently. Apparently the suppression or repression of leaflets may take effect at different stages of development. If the two lateral leaflets are completely suppressed at the beginning of a leaf's growth, a unifoliolate leaf results. If, on the other hand, one of the lateral leaflets starts to develop after a temporary repression while the other remains completely suppressed, a bilobed single leaflet (Figure 8*C*) with two main veins is formed; if both lateral leaflets begin to develop after a temporary repression, a trilobed leaflet (Figure 8*F*) with three main veins results. If one lateral leaflet is completely suppressed while the other develops normally, a bifoliolate leaf (Figure 8*E*) is formed. If one lateral leaflet starts to develop after a temporary repression while the other lateral leaflet has already begun to develop normally, the bifoliolate nature is maintained, but the terminal leaflet becomes bilobed (Figure 8*D*) and occasionally has two main veins similar to those seen in the single leaflet in Figure 8*C*. A few leaves with more than one leaflet are shown in the foreground of Figure 8*A*. In general they are smaller than the unifoliolate leaves and are much smaller than regular trifoliolate leaves from normal plants.

The inheritance of the unifoliolate character has not yet been studied. The plant did not flower after it was found in the nursery, and there was no indication that it had flowered earlier in the summer. At present, slips from this plant are being grown in the greenhouse under long day treatment in an attempt to induce flowering. The fact that the unifoliolate condition was found in only one plant in such a large open-pollinated population might signify that it is the expression of the recessive allelomorph of a gene for normal leaves. There was no indication that it was caused by a chromosomal change. The somatic number of chromosomes was found in root tips to be 32, the same as in normal

*A contribution from the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Northeastern States.

†The writer gratefully acknowledges the help of Mr. W. H. Brittingham in taking the photographs.

white clover plants, and no variation from the normal in chromosome morphology was observed. The chromosomes will be examined at meiosis as soon as the plant flowers.

The only condition of similar nature known to the author is that described by Lamprecht[1] for Pisum. In this case, the presence of leaves with a single leaflet was found to be inherited as a simple recessive character. They were always accompanied, however, by a multiple branching of the flower stalk resulting in several small head-like structures and by a tendency toward pistillody in the different floral elements, even in the small narrow leaf-like structures borne on the "head." In a later study,[2] he found the gene *uni* closely linked to *M*. He also noted in the segregates of a particular cross the influence of modifying factors since a large number of leaf-types were found, a few of which are characteristic for different genera in the Leguminosae.

Literature Cited

1. LAMPRECHT, H. Ein *unifoliata*—Typus von *Pisum* mit gleichzeitiger Pistilloidie. *Hereditas* 18:56-64. 1933-34.

2. LAMPRECHT, H. Das Gen *Uni* und seine Koppelung mit anderen Genen bei *Pisum*. *Hereditas* 18:269-296. 1933-34.

◆

The Hos Meet Industrialism

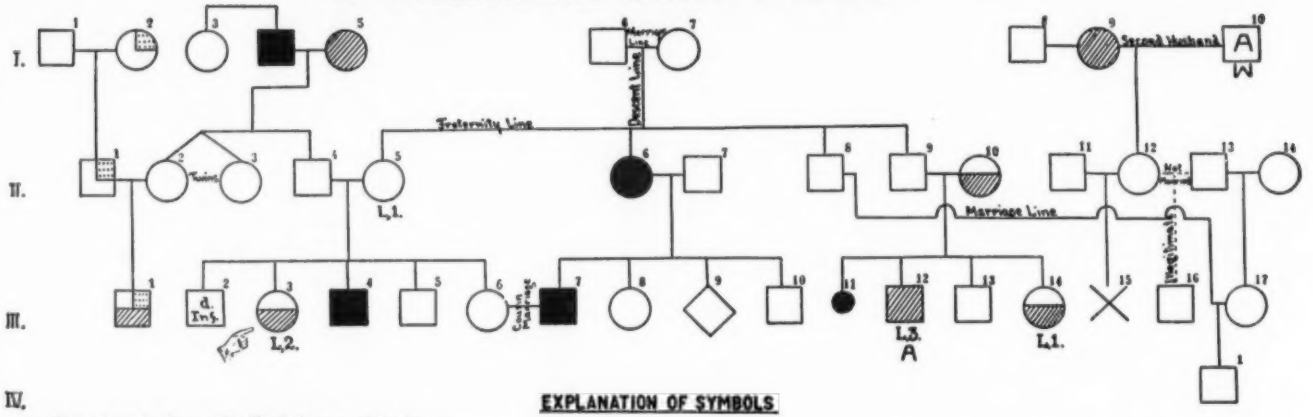
THE modification of culture by contact with foreign influences represents a field of study which is attracting increasing numbers of students. India offers innumerable examples of the process, although here the situation has been complicated by a tangled history of migrations and conquests.

In *A Tribe in Transition** Dr. Majumdar deals with the Ho people of the Singbhum district of India who number slightly more than 500,000. They are closely related to the Munda people of the Chota Nagpur Plateau. The Hos originally formed a primitive group, organized in village units and economically dependent on the neighboring forests. With the recent impact of industralized civilization their traditional customs have undergone a series of modifications. The new influences have penetrated to the Ho territory through channels created by governmental control, by the return of Ho laborers from centers of industrialization and by the agency of markets and fairs. Dr. Majumdar is here properly concerned mainly with ethnological data, but he does stress *en passant* the deterioration suffered by the male physique as a result of the breakdown of ancient controls on behavior. This work should be of interest to ethnogolists.

H. L. SHAPIRO
American Museum of Natural History

*D. N. MAJUMDAR. A Tribe in Transition. 216 pp., 3 appendices, index and bibliography. $4.00. Longmans - Green and Co., N. Y.

SAMPLE PEDIGREE CHART SHOWING THE MANNER OF CONSTRUCTION, AND THE USE OF STANDARD AND SPECIAL SYMBOLS.



EXPLANATION OF SYMBOLS

(a) Standard for all Pedigree Charts:

□=Male; ○=Female; ◇=Sex unknown; ●=Still-birth or Miscarriage; X=Children — number and sex unknown; ○○=Twins; d.Inf.=Died in Infancy; ☞ Points to the Propositus or central figure in the pedigree.

□—○=Parents.

□○□○○=Children.

Roman figures to the left indicate generations; Arabic figures locate individuals, (thus III.7 is the young man in the third generation who married his cousin).

The following letters, placed in or around the individual's pedigree symbol, are standard for certain traits: A, alcoholic; B, blind; D, deaf; E, epileptic; F, feebleminded; I, insane; M, migraineous; N, normal in reference to traits under consideration; Ne, neurotic; P, paralytic; Sx, sexually immoral; S, syphilitic; T, tuberculous; W, wanderer.

(b) Especially devised or selected for this particular pedigree-chart:

■ ● = Successful leaders in politics. ◪ ◔ = Extra thumb on right hand. L.1.=Highly successful author. L.3.=Little or no ability in literary efforts. ▨ ◍ = Superior in vocal music. ▤ ◍ = Medium attainment in vocal music.

To fit the particular family and traits (whether physical, mental or temperamental; good or bad) under consideration, invent special symbols, or select special letters (in addition to those standardized under (a) above) to be placed within or near the particular individual's pedigree symbol, to indicate particular traits and their degree of development.

Prepared by the International Commission of Eugenics. See *Journal of Heredity* for August 1926. Additional copies may be obtained from A.G.A.

Material for Demonstrating Salivary Gland Chromosomes

Interrelated demonstration material—microscopic slides, chromosome maps, lantern slides and literature—make giant chromosomes available for classroom use.

MICROSCOPIC SLIDES OF SALIVARY GLAND CHROMOSOMES

Slide of Normal Salivary Chromosomes of *D. melanogaster*, each	$2.25
Three Slides	5.00
Slide with drawing identifying all chromosomes in two figures (Female)	4.00*
Slide showing Y-chromosome (Male)	4.00
Slide showing synapsis of normal and inverted X-chromosome (loop)	4.00*
Slide showing figure of an autosomal inversion	4.00*
Slide showing synapsis of translocated and normal chromosomes	4.00*
Slide showing a small deficiency (deletion)	4.00*
Salivary chromosomes of *D. virilis*	2.25
Three Slides	5.00
Normal Somatic (ganglion) chromosomes of *D. melanogaster*	2.50
(Magnification of at least 1000 × necessary to view satisfactorily.)	
1 Slide of each of above (9 slides—6 drawings)	25.00

* Three or more slides, 1 of each kind @ $3.50 each. Three or more of same kind @ $3.00 each.

SALIVARY GLAND CHROMOSOME MAPS

Painter's cytogenetic map of the salivary chromosomes, 9½ by 18 inches, line-cut showing major chromosome details and the genes approximately located to end of 1934, mailed unfolded	$ .50
Bridges' reference map of the banding of the salivary chromosomes, 9½ by 25 inches, halftone on heavy coated paper, unfolded	1.00
Folded copies of Bridges' map, on lighter paper	.35
Hughes' map of Salivary Chromosomes of *Drosophila Virilis* (9½ by 18)	.75
1 copy each of Painter's, Hughes', and Bridges' maps	2.00

LANTERN SLIDES OF SALIVARY CHROMOSOME MATERIAL

†21-323.	Salivary chromosomes in the Nucleus	$ .75
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27-305.	Map of Salivary Gland Chromosomes of *D. Virilis*	.75
29-12.	Bridges' Revised Map of the S. G. X-Chromosome	.75
Set of 14 Lantern Slides		9.00

† Volume and page number of illustration in the JOURNAL OF HEREDITY.

ILLUSTRATED LITERATURE

"Symposium Reprint" on Salivary Gland Chromosomes, containing articles from the JOURNAL OF HEREDITY, 1934-1938, by Painter, Bridges, Metz, Mackensen and Hughes—four Salivary Gland Chromosome maps, 15 illustrations, 54 pages $3.00

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